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Original Paper

# An Epidemiological Investigation of Hospital Infections Caused by MRSA and Their Prevention

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Abstract

To reexamine measures for preventing of hospital infections caused by medical staff and in order to decrease hospital infection rates, MRSA inhabitation and transmission patterns were investigated in two facilities (hospitals X and Y), by gene analysis using pulse field gel electrophoresis (PFGE). The DNA patterns of MRSA isolated from patients and from the fingers and uniforms of nurses, who cared for the patients, were identical, indicating inadequate standard precaution (SP) practice, including proper hand washing and gown technique. The results were reported to the facilities, and new infection control procedures were instituted. After implementation of the revised guidelines, the same survey was repeated in the same wards. In hospital X, no MRSA strain was isolated from individuals other than the MRSA patients. The new procedures based on the epidemiological investigation by PFGE were effective in preventing of hospital infections. In contrast, hospital Y was unable to institute systematic measures for infection control, and contamination and transmission of MRSA appeared in the second investigation, showing that the survey results had no effect. Effective intervention for promotion of systematic, rational measures for infection control remains an issue at this hospital.

## Introduction

Methicillin resistant *staphylococcus aureus* (MRSA) has attracted attention as an etiologic agent of hospital infection since the 1980s [1,2]. The Ministry of Health and Welfare prepared a manual for the control of infections including MRSA in 1985[3]. In Japan, the 'Law concerning the prevention of infections and medical care for patients of infection (New Law for Infectious Diseases)' issued in 1999, categorized, MRSA as a Type 4 infection[4]. It was predicted that MRSA, along with vancomycin-resistant *enterococcus* (VRE) and drug-resistant *pseudomonas aeruginosa*, would be important in efforts to control the spread of infections caused by in the $21^{st}$ century.

The objective of controlling infections in hospitals is to protect patients and medical staff from incidental infections and to promote high-quality care for patients and patient's families [5]. To accomplish this

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objective, measures such as evidence-based precaution (EBP), intervention to improve compliance for infection control measures, and monitoring before and after implementation of the measures are important [6]. Since the cause of most hospital infections is by contact with medical staff [7,8], it is necessary to identify and interrupt the transmission pathway of the etiological microorganisms.

To improve the quality of health services, the spread of MRSA from patient to nurses was surveyed in two medical facilities, and the dissemination of specific MRSA strains was studied. To investigate transmission pathways, the DNA band patterns of MRSA were analyzed by pulse field gel electrophoresis (PFGE) [9,10], and homology among the strains was determined. The results were sent back to the medical facilities, where revised measures for hospital infection control were investigated. After the measures had been implemented, the same survey was performed in the same wards of each facility to evaluate effectiveness.

## Materials and Methods

Prior to the study, agreement was obtained from the responsible physicians, their patients, and the patients' families. The survey was performed twice in Hospitals X and Y, with an interval of 8–11 months between the first and second surveys.

1. Sample collection

1) Samples from MRSA patients

For samples from MRSA patients, swabs were taken from wounds, feces, urine, and sputum and smeared on a mannitol salt agar medium.

2) Environment at samples

Using Replicated Organism Detection and Counting (RODAC) dishes (Becton Dickinson, USA) containing 17 ml of mannitol salt agar, samples were collected from floors, bed sheets, and bedside tables by the stamp method.

3) Samples from nurses

Using sterilized swabs moistened with sterilized physiological saline, samples were collected from the nasal cavities of the nurses and smeared on mannitol salt agar medium. Also the fingerprint region of the fingers of the right hand were slightly compressed on mannitol salt agar medium before and after hand washing. Samples were obtained on RODAC® stamp medium from two abdominal regions of nurses' clothes.

2. Identification of sample bacteria

The samples collected on mannitol salt agar medium plates were incubated at 37 ℃ for 48 hours, and colonies with yellowish color change were isolated and seeded in heart infusion agar medium (HIA, Nissui Pharmaceutical Co., Ltd.). The pure culture colonies were subjected to gram staining, catalase test, and coagulase test, after which bacteria were identified using a simple fixation kit (API system®, BIO MARIEUX, France).

3. Determination of MRSA

Isolated samples identified as *staphylococcus aureus* were subjected to a drug sensitivity test. Using Sensi-Disc® (Becton Dickinson, USA), samples with inhibition zones of 9 mm or less for methicillin (DMPPC) and 10 mm or less for oxacillin (MPIPC) were determined to be MRSA.

4. Typing by PFGE

To investigate homology among the isolates determined as MRSA, DNA fingerprinting was performed by PFGE. PFGE was performed according to the Gene Path® Group I Reagent Kit Instruction Manual (BIO-RAD, USA). Chromosomal DNA was cleaved with restriction enzyme *Sma I*, elctrophoresed, stained, and photographed. For electrophoresis, counterclamped homogenous electric field gel electrophoresis (CHEF) (CHEF-DRII®, BIO-RAD, USA) was used. The electrophoresis conditions were: pulse time, 5–45 seconds; voltage, 200 V; electrophoresis time, 22 hours. For molecular weight markers, λ DNA ladder was used.

Table 1 Number of samples and MRSA isolates in hospital X

| | | 1st survey | | 2nd survey | |
|---|---|---|---|---|---|
| | | Number of samples | Number of MRSA isolates | Number of samples | Number of MRSA isolates |
| MRSA patient | | | | | |
| | Wound | 2 | 2 | 1 | 1 |
| | Sputum | 1 | 0 | 2 | 1 |
| | Feces | 1 | 0 | | |
| | Urine | 1 | 0 | 1 | 0 |
| Environment | | | | | |
| | Floor | 44 | 4 | 46 | 0 |
| | Sheets/hospital clothes | 14 | 3 | 10 | 0 |
| | Over table | 7 | 1 | 14 | 0 |
| | Doorknob | 10 | 0 | 10 | 0 |
| Nurses | | | | | |
| | Nasal cavity | 8 | 0 | 21 | 0 |
| | Uniforms | 14 | 2 | 8 | 0 |
| | Fingers | 10 | 4 | 16 | 0 |
| Total | | 112 | 16 | 129 | 2 |

Table 2 MRSA isolation sites and their DNA patterns in hospital X

| MRSA isolate No. | MRSA isolation site | DNA pattern |
|---|---|---|
| 1 | Patient A abdominal wound | $O_1$ |
| 2 | Patient A sheets (head region) | $O_1$ |
| 3 | Patient A floor under the bed | $O_1$ |
| 4 | Patient B floor under the bed | $O_2$ |
| 5 | Patient C floor under the bed | $O_3$ |
| 6 | Nurse station table | $O_1$ |
| 7 | Nurse D fingers (after care for patient A) | $O_1$ |
| 8 | Patient A sheets(leg region) | P |
| 9 | Patient A bedsore | $Q_1$ |
| 10 | Patient A hospital clothes | P |
| 11 | Patient B floor under the bed | $Q_2$ |
| 12 | Nurse E fingers(after care for Patient A) | $O_1$ |
| 13 | Nurse E fingers(after hand washing after care for Patient A) | P |
| 14 | Nurse E uniform(before care) | P |
| 15 | Nurse F uniform(after care for Patient A) | P |
| 16 | Nurse D fingers (after hand washing after care for Patinet A)) | P |

*Patient A was infected with MRSA

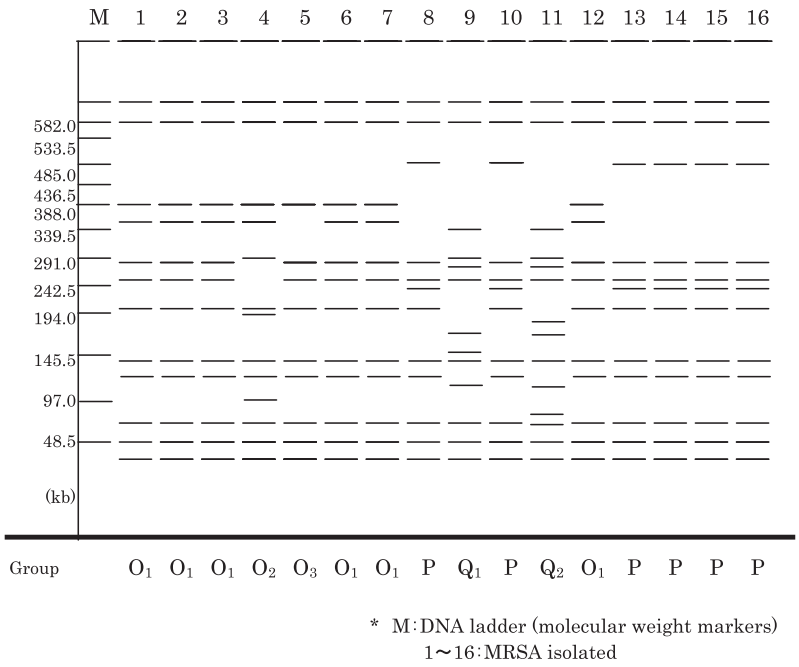


Fig. 1 PFGE schematic patterns (*Sma I* digestion) and grouping of MRSA isolates in hospital X (first survey)

## Results

1. Results of survey in hospital X

1) First survey

(1) MRSA isolation sites

In the first survey, one MRSA patient (Patient A) was in a single room. Among five samples from this patient, two MRSA strains were isolated. From 75 samples from the environment and 32 samples from nurses, eight and six strains of MRSA were isolated, respectively, a total of 16 isolates (Table 1).

The sites from which the 16 MRSA strains were isolated are shown in Table 2. MRSA was isolated from an abdominal wound and bedsore in Patient A, but none was isolated from sputum, feces, or urine. In the environment, MRSA was isolated from the bed sheets, hospital clothes, and floor under the bed of Patient A, floors under the beds of non-MRSA Patients B and C, and a table in the nurse station. MRSA was also isolated from the nursing uniform of Nurse E before she cared for patients, and from the nursing uniform of Nurse F after caring for Patient A. MRSA was isolated from the fingers of both Nurse E and nurse D after they had cared for Patient A and washed their hands.

(2) PFGE patterns of MRSA isolates

A schematic presentation of PFGE DNA patterns and their classification are shown in Fig. 1. MRSA isolation sites and DNA patterns are shown in Table 2. Based on the cleavage patterns of the 16 MRSA isolates, DNA in lanes 1, 2, 3, 6, 7, and 12 were the same type and related to those in lanes 4 and 5. These were designated as Groups $O_1$, $O_2$, and $O_3$, respectively. DNA in lanes 8, 10, 13, 14, 15, and 16 were the same type and designated as Group P. They were related to the DNA in lanes 9 and 11 which were designated as Groups $Q_1$ and $Q_2$. DNA of the MRSA strain derived from the abdominal wound of Patient A (lane 1) was identical to those from the bed sheets and floor under the bed of Patient A (lanes 2 and 3), fingers of Nurses D and E who cared for Patient A(lanes 7 and 12), and the nurse station table (lane 6). DNA of these strains were related to those from the floors under the beds of Patients B and C (lanes 4 and 5) (O pattern). The strain derived from the bedsore of Patient A (lane 9) was related to those from the

floor under the bed of Patient B (lane 11). However, the origin was unclear for the six strains showing the P pattern: those from the sheets and hospital clothes of Patient A (lanes 8 and 10), the fingers of Nurses E and D after they washed their hand (lanes 13 and 16), and the nursing uniforms of Nurses E and F (lanes 14 and 15).

(3) Infection control

The above findings were reported to hospital X, and a hospital infection control committee meeting was held. Problems found in the previous test were discussed, and the following measures were implemented:

(a) Distribution of a booklet of "Standard precautions" to each ward.

(b) Promotion of standard precautions.

- Implementating correct hand washing procedures (poster presentation)
- Distributing liquid soap (Shabonet®) and paper towels to each hand washing site.
- Promoting disinfecting of hands using Welbath® before and after caring for patients.
- Wearing gloves when contacting body fluids of patients, and washing hands after removing gloves.
- Wearing masks when specimens may splash.
- Wearing protective clothing when contamination of clothes is likely.
- Proper handling of contaminated linen to avoid contamination of other patients and the environment.
- Isolating patients who may be sources pf contamination.

(c) Informing hospital staff by circulating the test results in the wards.

(d) Revision of the manual

(e) Instituting an education program

- Instruction of all nursing staff by infection control nurses.
- Internal training meeting
- Infection control nurses making rounds on wards.

(f) A survey of bacterial contamination of the environment.

2) Second survey

The second survey was performed 11 months after the first. In the second survey, there were two MRSA patients in single rooms. Samples were collected from the sputum and bedsore of one patient and sputum and urine of the other (Table 1). Among the four samples, one strain of MRSA was isolated from the wound and one from the sputum. From the surrounding areas, 80 samples were collected from floor, bed sheets, bedside tables, and doorknobs, but no MRSA was isolated from any of the samples. In addition, 45 samples were collected from the nasal cavities, fingers, and nursing uniforms of nurses, but no MRSA was isolated from any of the samples (Table 1).

2. Results of survey in hospital Y

1) First survey

(1) MRSA isolation sites

There were three MRSA patients (a, b, c) at the time of the survey. Patients a and b stayed in single rooms and Patient c in a double room. MRSA was detected in the sputum, bedsore, feces, and urine of all three patients. As shown in Table 3, among three sputum samples and one each of wound, feces and urine collected from these three patients, one MRSA strain was isolated from sputum from each patient. Among

178 samples collected from the surrounding areas such as floors, bedclothes, and headrests, 14 strains of MRSA were isolated. Among 57 samples derived from the nasal cavity, fingers, and uniform of nurses, eight strains were identified making a total of 25 strains of MRSA that were isolated.

The sites from which the 25 MRSA strains were isolated are shown in Table 4 (No. 1–25). MRSA was isolated from the sputums of MRSA patients a, b, and c; the floor under the bed of Patient a; headrest, bed sheet, and bedside table of Patient b; and the floor under the bed, bedside table, and a bed sheet of Patient c. MRSA was also isolated from the headrest and floor under the bed of non-MRSA Patient d, who shared the same room with Patient c, the toilet near Patient c's room, the nasal cavities of Nurses e, g, and h, the fingers of Nurses e, f, and h before giving care and the uniforms of Nurses g and h.

Table 3 Number of samples and MRSA isolates in hospital Y

| | 1st survey | | 2nd survey | |
|---|---|---|---|---|
| | Number of samples | Number of MRSA isolates | Number of samples | Number of MRSA isolates |
| MRSA patient | | | | |
| Wound | 1 | 0 | 1 | 0 |
| Sputum | 3 | 3 | 5 | 5 |
| Feces | 1 | 0 | 1 | 0 |
| Urine | 1 | 0 | 1 | 0 |
| Environment | | | | |
| Floor | 62 | 4 | 58 | 4 |
| Sheets/hospital clothes | 48 | 3 | 48 | 2 |
| Over table | 25 | 3 | 25 | 1 |
| Headrest | 26 | 4 | 26 | 2 |
| Doorknob | 17 | 0 | 11 | 1 |
| Nurse | | | | |
| Nasal cavity | 19 | 3 | 20 | 3 |
| Uniforms | 19 | 2 | 20 | 0 |
| Fingers | 19 | 3 | 20 | 1 |
| Total | 241 | 25 | 236 | 19 |

(2) PFGE patterns of MRSA isolates

A schematic presentation of the PFGE MRSA DNA patterns and their classifications are shown in Fig. 2. The MRSA isolation sites and DNA patterns are shown in Table 4. Nine DNA patterns were observed in the 25 MRSA isolates, and were even designations from A to I. The DNA in lanes 5, 6, 13, 14, and 22 were of the same type ($E_1$) and related to those in lanes 10 and 12 ($E_2$: E pattern ), showing a commonality among strains derived from the sputum of MRSA patient b (lane 6), the floor under the bed of MRSA patient a (lane 5), the bedside table and bed sheets of MRSA patient c (lanes 13 and 14), and nursing uniform of Nurse g (lane 22). They were also related to isolates from the floor under the bed of MRSA patient c (lane 12) and bed sheets of MRSA patient b (lane 10). DNA in lanes 11, 18, and 19 were of the same type, showing a relationship among the sputum of MRSA patient c, and isolates from the nasal cavity and fingers of Nurse e (G pattern). The DNA of isolates from the nasal cavity of Nurse g (lane 21) were similar to those from a headrest (lane 2) and floor under the beds (lane 15) of non-MRSA patients (B pattern). Furthermore, the same type was isolated from the nasal cavity, fingers, and uniform of Nurse h (lanes 23, 24, and 25) (I pattern).

(3) Infection control

The above findings were reported to hospital Y, and problems and measures for improving previous infection control procedures were investigated. Use of adhesive mats placed at the entrance of the rooms of MRSA patients and changing slippers for exclusive use were re-examined. The following three measures

Table 4 MRSA isolation sites and their DNA patterns in hospital Y

| MRSA strain No. | MRSA isolation site | DNA Pattern |
|---|---|---|
| 1 | Room 14 headrest | A |
| 2 | Room 9 headrest | B |
| 3 | Room 8 over table | C |
| 4 | Room 6 Patient A sputum | D |
| 5 | Room 6 Patient A floor under the bed | $E_1$ |
| 6 | Room 5 Patient B sputum | $E_1$ |
| 7 | Room 5 Patient B headerest | F |
| 8 | Room 5 Patient B sheets(head region) | F |
| 9 | Room 5 Patient B over table | F |
| 10 | Room 5 Patient B sheets(leg region) | $E_2$ |
| 11 | Room 3 Patient C sputum | $G_1$ |
| 12 | Room 3 Patient C floor under the bed | $E_2$ |
| 13 | Room 3 Patient C over table | $E_1$ |
| 14 | Room 3 Patient C sheets (head region) | $E_1$ |
| 15 | Room 3 Patient D floor under the bed | B |
| 16 | Room 3 Patient D headrest | H |
| 17 | Women's toilet floor | C |
| 18 | Nurse E nasal cavity | $G_1$ |
| 19 | Nurse E fingers(before care) | $G_1$ |
| 20 | Nurse F fingers(before care) | H |
| 21 | Nurse G nasal cavity | B |
| 22 | Nurse G uniform | $E_1$ |
| 23 | Nurse H nasal cavity | I |
| 24 | Nurse H fingers(before care) | I |
| 25 | Nurrse H uniform | I |
| 26 | Room 12 doorknob | $G_2$ |
| 27 | Room 4 PatientI sputum | J |
| 28 | Room 3 Patient C floor under the bed | $G_1$ |
| 29 | Room 3 Patient C sputum | $G_2$ |
| 30 | Room 3 Patient J sputum | K |
| 31 | Room 2 sheets (head region) | $G_2$ |
| 32 | Room 2 headrest | $G_2$ |
| 33 | Room 2 over table | $G_2$ |
| 34 | Room 1 Patient K floor under the bed | $G_2$ |
| 35 | Room 1 Patient K sheets (head region) | $G_2$ |
| 36 | Room 1 Patient K headrest | $G_2$ |
| 37 | Room 1 Patient K sputum | $G_2$ |
| 38 | Room 1 Patient L sputum | $G_2$ |
| 39 | Sanitation room, floor | $G_2$ |
| 40 | Nurse station, floor | L |
| 41 | Nurse N nasal cavity | $G_3$ |
| 42 | Nurse G fingers(before care) | $G_3$ |
| 43 | Nurse G nasal cavity | $E_1$ |
| 44 | Nurse F nasal cavity | H |

*Patient A,B,C,I,J,K,and L were infected with MRSA

were agreed upon as the primary objectives of the wards.

(a) Compliance with strict of hand washing procedures by staff.
(b) Use of Welbath® in places lacking hand washing apparatus.
(c) Explaining and promoting hand washing to visitors.

However, since the infection control committee of the hospital did not have the time to monitor procedures, it was decided to continue use of the previous manual.

2) Second survey

(1) MRSA isolation sites

The second survey was performed eight months after the first. There were five MRSA-infected patients (c, i, j, k, l), with Patients c and j in oneroom, Patients k and l in another, and Patient i in a single room. MRSA was detected in the sputum of all five patients, the bedsore of Patient c and the feces and urine of Patient j. Patient c had been continuously hospitalized since the time of the first survey and was in a critical state requiring frequent suctioning of sputum.

As shown in Table 3, five MRSA strains were isolated from the sputum of the five MRSA patients, 10 from 168 environment samples, and four from 60 samples from nurses, a total of 19 MRSA isolates.

The sites from which the 19 strains were isolated are shown in Table 4 (No. 26–44). MRSA was isolated from the sputum (No. 29, 27, 30, 37, 38) of the five MRSA patients (c, i, j, k, l), the floor under the bed of Patient c, and the bed sheets, headrest, and floor under the bed of Patient k. MRSA was also isolated from doorknobs, bed sheets, head rests, and bedside tables in rooms of non-MRSA patients, sanitation room, the floor of the nurses' station, the nasal cavity of Nurses n, g, and f, and the fingers of Nurse g.

(2) PFGE patterns of MRSA isolates

A schematic presentation of the DNA patterns and their classifications are shown in Fig. 3. MRSA isolation sites and DNA patterns are shown in Table 4. The DNA patterns of the 19 isolates were classified into six patterns and designated as E, G, H, J, K, and L.

DNA in lanes 26, 29, and 31–39 were the same type ($G_2$) and the DNA in lane 28 was related to those in lanes 41 and 42 ($G_1$, $G_3$), showing that MRSA derived from the sputum of Patients c, k, and l were the same type. This type was also found in areas other than the rooms of these patients. These G patterns were related to the DNA pattern ($G_1$) isolated from the sputum of Patient c and the nasal cavity and fingers of Nurse e in the first survey. Furthermore, MRSA isolated from the nasal cavity of Nurse g (lane 43) was similar to the strains from the sputum of Patient b and nursing uniform of Nurse g isolated in the first survey ($E_1$).

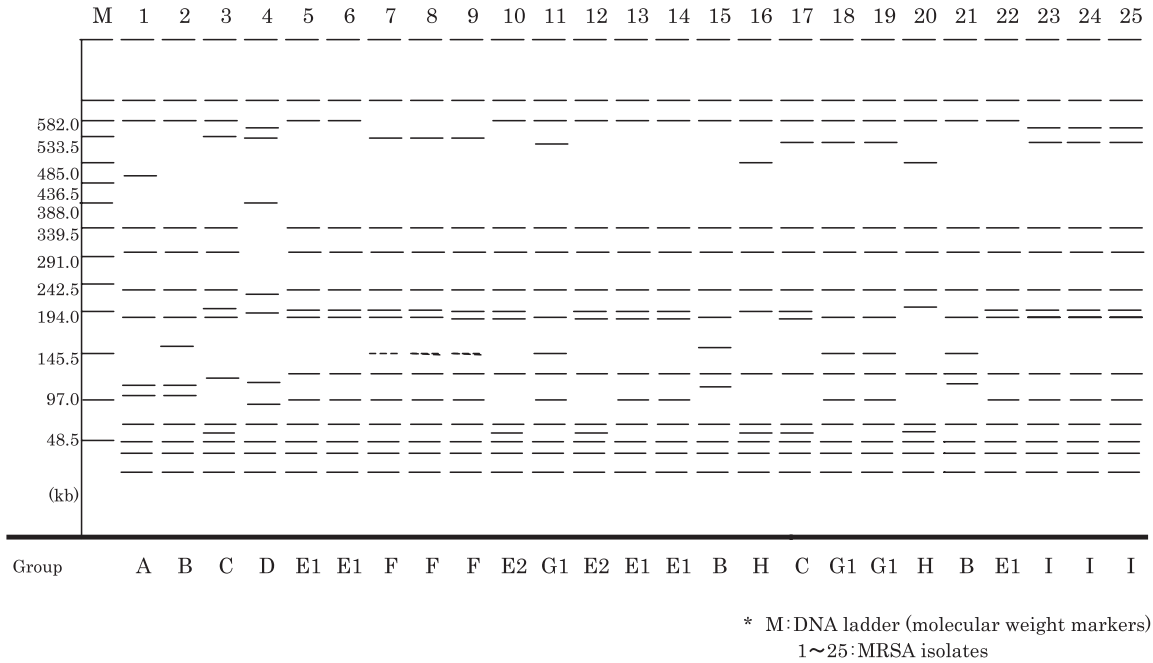


Fig. 2 PFGE schematic patterns (*Sma I* digestion) and grouping of MRSA isolates in hospital Y (first survey)

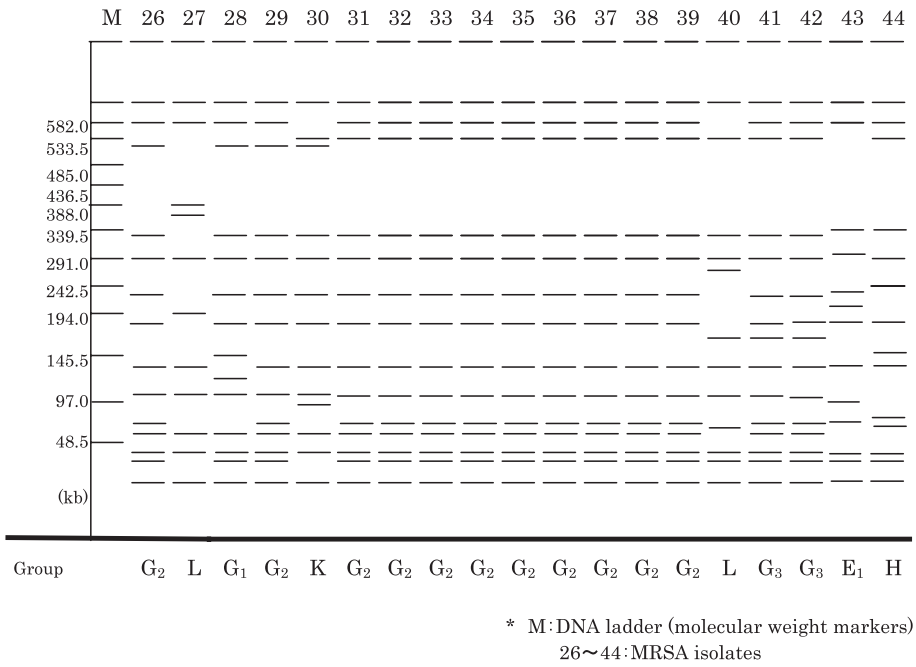


Fig. 3 3 PFGE schematic patterns (*Sma I* digestion) and grouping of MRSA isolates in hospital Y (second survey)

## Discussion

In Japan, it is anticipated that the number of immunocompromised patients will rise with the increase in the elderly population due to advances in medical care. This means that reinforcement of infection control in medical facilities is urgently needed. The basic strategy against hospital infections is the Standard Persuasions (SP) of the CDC guideline [11]. However, SP is not extensively practiced in Japan [12], and the compliance rate is low in Western countries[13,14]. In this study, we surveyed inhabitation of MRSA and its prevalence among nurses in two medical facilities and performed a molecular epidemiological analysis of the transmission pathway of specific MRSA strains. The results of the survey were reported to the medical facilities so that they could institute more effective compliable infection control. Several months later, the same survey was performed and the effect of implemented measures was analyzed for each facility.

In the first survey in hospital X, the same type of MRSA was isolated from the wound of the MRSA patient, the fingers of nurses after providing care, and the nurse station. Also, related types were isolated from two rooms of non-MRSA patients (O pattern). The DNA (P pattern) isolated from the bed clothes of MRSA patients was similar to those from the nurses' uniforms before and after they cared for the MRSA patient and the nurses' fingers before and after hand washing (Table 2, Fig. 1).

These findings indicated that MRSA was transmitted from the patient via the fingers and uniforms of nurses. The causes were considered to be as follows: 1) Nurses cared for the MRSA patient without wearing protective clothes and masks, 2) nurses cared for the MRSA patient with bare hands, and 3) inadequate hand washing with soap and tap water after giving care. Based on the survey results, the infection control committee of hospital X held a training meeting for all staff and thoroughly promoted SP, the basic strategy against hospital infection [11].

In the second survey performed 11 months after the first survey, MRSA was isolated from the sputum of the MRSA patient, but no MRSA was isolated from the environment or nurses (Table 1). This may have

been due to feedback of the survey data which motivated the medical staff to investigate evidence-based precaution (EBP) [15] and made all medical staff share the objective of being aware and responsible for infection control. The entire organization cooperated to improve compliance to infection control [16,17,18].

In the first survey in hospital Y, MRSA strains having the same DNA patterns (E and G patterns) as those derived from the sputum of MRSA Patients b and c were isolated from the rooms of non-MRSA patients and the nasal cavity, fingers, and nursing uniforms of nurses. Isolated with a similar DNA pattern were found in the nasal cavity of Nurse g, isolates from Patient c, and those from a headrest in a room far from Patient c's room (B pattern) (Table 4, Fig. 2). These findings indicated that contamination was transmitted via the fingers and uniforms of nurses. The cause was shown to be inadequate SP including nurses' hand washing and gown technique. This result was reported to hospital Y, but the hospital failed to take systematic measures to prevent the spread of infections. The hospital agreed in principle to comply with 'complete performance of hand washing'.

In the second survey, among 228 samples excluding those from MRSA patients, 14 MRSA strains were isolated from doorknobs, bed sheets, headrests, and bedside tables (Tables 3 and 4). Eight of these isolates had the same DNA pattern (G pattern), which was similar to those derived from the sputum of Patients c, k, and l (Table 4, Fig. 3). The condition of MRSA Patient c was more critical than at the time of the first survey and the patient required frequent suctioning sucking of sputum. This may have resulted in the cross contamination of infection by patient-nurse contact. The same or related MRSA strains as those found in the first survey were isolated in the second survey, suggesting that the MRSA inpatients were the sources of infection or setting in the environment [19]. Although hospital Y had agreed to improve hand washing techniques based on the data of the first survey, the infection control committee of the hospital did not systematic ally correct existing problems or investigate new solutions. No concrete action objectives were clarified for the medical staff, which may have resulted in the spread of infection. MRSA is an indigenous bacteria and a small amount of the bacteria exists in the nasal cavities and fingers of 3–5% of healthy individuals. However, MRSA is very likely to induce microbial substitution in immunocompromized hosts and can start cross infections in hospitals, a fact that medical professionals should keep in mind. To prevent hospital infections and assure quality care for patients and their families, in addition to complying with infection control and SP procedures, effective intervention for systematic improvement and monitoring should be a priority.

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